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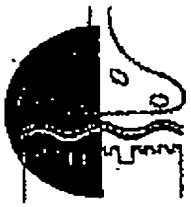

XIth INTERNATIONAL SYMPOSIUM ON CHOLINERGIC MECHANISMS- FUNCTION AND DYSFUNCTION & 2nd MISRAHI SYMPOSIUM ON NEUROBIOLOGY

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SOME BASIC RULES GOVERNING OLIGOSACCHARIDE-DEPENDENT CIRCULATORY RESIDENCE OF GLYCOPROTEINS ARE REVEALED BY MALDI-TOF MAPPING OF THE MULTIPLE N-GLYCANS ASSOCIATED WITH RECOMBINANT BOVINE ACETYLCHOLINESTERASE

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Pharmacokinetic studies of recombinant bovine acetylcholinesterase (rBoAChE), revealed that this enzyme is cleared more rapidly than the native serum-derived FBS-AChE. Extensive MALDI-TOF analysis of sialylated and desialylated glycans purified from rBoAChE revealed that these are comprised of a complex array of diverse structures differing in branching, monosaccharide substitutions and relative abundances. The exact structures of the different glycans were confirmed by a series of exoglycosidase treatments followed by MALDI-TOF analysis. The most prevalent structure was the biantennary fucosylated form, (Man)3-[GlcNAc]2-([]-gal)2-Fuc, which constitutes approximately 40-50 percent of the total glycans, 20-30% of the glycans were of the triantennary form, while tetraantennary glycans were present at very low levels. Most importantly, the glycans of rBoAChE were found to be heavily unsialylated, containing 4-5 terminally exposed β-gal per enzyme subunit.

To allow efficient sialylation, rBoAChE was produced in an engineered HEK-293 cell line which expresses high levels of recombinant sialyltransferase. MALDI-TOF analysis of the glycans of rBoAChE produced in these cells demonstrated that the vast majority of these glycan forms were now highly sialylated. Pharmacokinetic studies of highly-sialylated rBoAChE established that this enzyme was retained in the circulation for extended periods of time, as compared to unsialylated rBoAChE. These studies emphasize the pivotal role of glycan sialylation in determining the circulatory fate of cholinesterases, and provide the basis for detailed determination of their glycan structures.

This work was supported in part by the U.S. Army Research and Development Command, Contracts DAMD17-96-C6086 and DAMD17-00-C0021 (to A.S.)

EFFECT OF POST-TRANSLATION MODIFICATIONS OF HUMAN ACETYLCHOLINESTERASE ON ITS CIRCULATORY RESIDENCE

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Homogeneous preparations of rHuAChE differing in their oligomerization status were generated: (a) monomer - represented by the oligomerization-impaired C580A-rHuAChE mutant (b) dimeric wild-type (WT) and (c) tetramers of WT-rHuAChE generated *in-vitro* by complexation with a synthetic ColQ-derived Proline Rich Attachment Domain (PRAD) peptide. Three different series of each of these five oligomeric preparations were produced: 1) partially sialylated - derived from HEK-293 cells; 2) fully sialylated – derived from HEK-293 engineered cells expressing high levels of sialyltransferase; 3) desialylated – following treatment with sialidase to quantitatively remove sialic acid termini. The oligosaccharides associated with each of the various preparations were extensively analyzed by MALDI-TOF. With the enzyme preparations comprising the fully sialylated series a clear linear relationship between oligomerization and circulatory mean residence time (MRT) was observed. Thus, monomers, dimers and tetramers exhibited MRTs of 110, 195, and 740 min respectively. As the level of sialylation decreased, this differential behavior became less pronounced, and eventually following desialylation all oligomers had the same MRT (5 min). These observations suggest that multiple removal systems contribute to the elimination of acetylcholinesterase from the circulation. The studies presented here demonstrate also that by combined modulation of sialylation and tetramerization it is possible to generate a rHuAChE displaying a circulatory residence exceeding that of all other known forms of native or recombinant human AChE.

This work was supported in part by the U.S. Army Research and Development Command, Contract DAMD17-00-C0021 (to A.S.)

CHANGES IN NEURONAL CHOLINERGIC RECEPTOR BINDING SITES AT DIFFERENT AGES IN TRANSGENIC MICE OVEREXPRESSING HUMAN ACETYLCHOLINESTERASE

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Deficits in the cholinergic circuits of the cortex brain are observed in neurodegenerative disorders like Alzheimer's and Parkinson's disease. An overexpression of neuronal human acetylcholinesterase (AChE) in mice causes alteration in the cholinergic transmission. The objective of this study was to investigate how an overexpression of AChE activity influences the plasticity of cholinergic neurons, particularly the nicotinic and muscarinic receptor subtypes. AChE transgenic (Tg+) mice at different ages, from 3 days old up to 1 year, were compared to age-matches non-transgenic (Tg-) mice. The nicotinic receptor binding sites were quantified, in the cortex and the striatum using [^{3}H]cytisine (alpha4beta2) and in the cortex and the hippocampus using [^{125}I]alpha-bungarotoxin (alpha7). In addition, muscarinic receptor binding sites were quantified, in the cortex and the striatum using [^{3}H]AF-DX-384 (M2) and in the cortex using [^{3}H]pirenzepine (M1). A significantly increased [^{3}H]cytisine binding were found in the cortex and the striatum in AChE Tg+ mice in comparison to Tg- mice in various age groups. No major alteration in [^{125}I]alpha-bungarotoxin binding sites were observed in Tg+ compared to Tg- mice. However, an up-regulation of [^{3}H]AF-DX-384 binding sites were found in the striatum in AChE overexpressing mice at 3 month of age compared to age-matched control mice while no change in [^{3}H]pirenzepine binding sites were detected at any age. The increase in alpha4beta2 and M2 receptor binding sites observed in AChE Tg+ mice was found at all ages and thus not influenced by aging processes.

TRANSGENIC OVEREXPRESSION OF READTHROUGH ACETYLCHOLINESTERASE (AChE-R): DISTRIBUTION OF AChE-R AND cFOS IN BRAIN IN RELATION TO BEHAVIOR

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Acute stress induces a cascade of events, which involves increased release of acetylcholine and feedback activation of acetylcholinesterase gene expression, shifting into the 'readthrough' acetylcholinesterase variant AChE-R which contributes to downregulating stress-induced cholinergic excitability. To explore whether chronic elevation of AChE-R affects behavioral and physiological functions, we employ FVB/N transgenic (Tg) mice over-expressing human AChE-R. The present report correlates behavioral and physiological functions with brain distribution of AChE-R and of cFOS, an immediate early gene that is responsive to stress. Tg AChE-R mice appeared healthy but their body weight was lower compared to FVB/N mice. Low body weight correlated with appearance of AChE-R-filled neurons in the lateral hypothalamic area and with increase in c-FOS positive cells in lateral and ventromedial hypothalamus. Compared to FVB/N mice, Tg-AChE-R mice were significantly impaired in learning in a social choice maze. This was correlated with appearance of AChE-R-filled neurons and c-FOS positive cells in the hippocampal dentate gyrus. Additional mice, were implanted with transmitters and their motor activity recorded in the home-cage. Increased motor activity in Tg-AChE-R mice correlated with appearance of AChE-R-filled neurons and c-FOS positive cells in striatum, parietal and neocortical cortex. We propose that chronic elevation of AChE-R alters regulation of neuronal excitability in several brain regions that, in turn, may contribute to the alterations in behavioral and physiological functions under conditions of chronic stress.